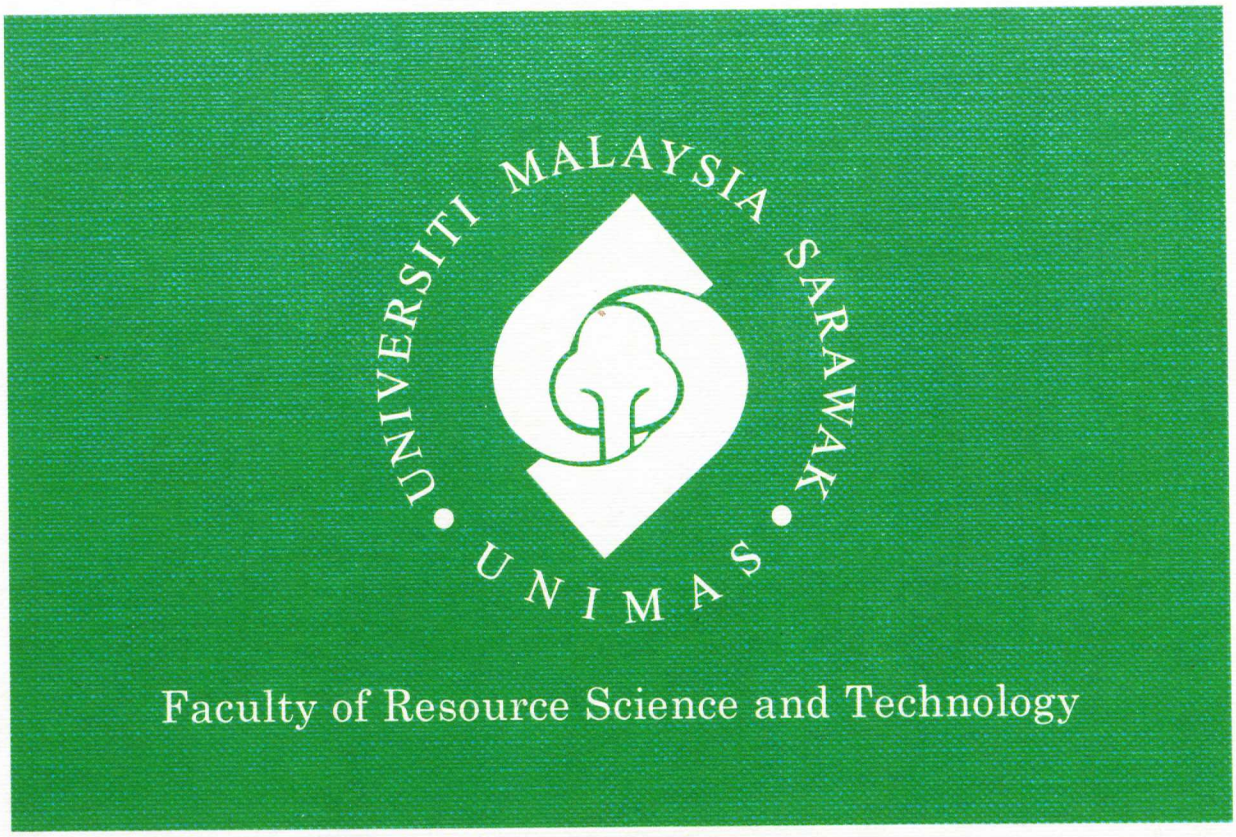

SYSTEMATICS OF *HIPPOSIDEROS GALERITUS* USING CYTOCHROME C OXIDASE I GENE, MORPHOLOGY AND ECHOLOCATION CALL

Tan Jo Ann

Bachelor of Science with Honours
(Animal Resource Science and Management)
2015

UNIVERSITI MALAYSIA SARAWAK

Grade: _____

Please tick (√)

Final Year Project Report [√]

Masters [ ]

PhD [ ]

DECLARATION OF ORIGINAL WORK

This declaration is made on the 25 day of June 2015 ~~2012~~.

Student's Declaration:

I TAN JO ANN, 39028, Faculty of Resource Science and Technology

(PLEASE INDICATE STUDENT'S NAME, MATRIC NO. AND FACULTY) hereby declare that the work entitled Systematics of Hipposideros galeritus using cytochrome c oxidase I gene, morphology and echolocation call is my original work. I have not copied from any other students' work or from any other sources except where due reference or acknowledgement is made explicitly in the text, nor has any part been written for me by another person.

25/6/2015
Date submitted

TAN JO ANN (39028)
Name of the student (Matric No.)

Supervisor's Declaration:

I FAISAL ALI ANWARALI KHAN (SUPERVISOR'S NAME) hereby certifies that the work entitled ~~SYSTEMATICS OF HIPPOSIDEROS GALERITUS USING CYTOCHROME OXIDASE I GENE, MORPHOLOGY AND ECHOLOCATION CALL~~ (TITLE) was prepared by the above named student, and was submitted to the "FACULTY" as a * partial/~~full~~ fulfillment for the conferment of B.Sc. (Hons) Animal Resource Science and Management (PLEASE INDICATE THE DEGREE), and the aforementioned work, to the best of my knowledge, is the said student's work.

Received for examination by: _____ Date: 25/6/2015

(Name of the supervisor)

Dr. Faisal Ali Anwarali Khan
Pensyarah
Fakulti Sains dan Teknologi Sumber
UNIVERSITI MALAYSIA SARAWAK
94300 Kota Samarahan

I declare that Project/Thesis is classified as (Please tick (√)):

☐ **CONFIDENTIAL** (Contains confidential information under the Official Secret Act 1972)*
☐ **RESTRICTED** (Contains restricted information as specified by the organisation where research was done)*
☑ **OPEN ACCESS**

Validation of Project/Thesis

I therefore duly affirm with free consent and willingly declare that this said Project/Thesis shall be placed officially in the Centre for Academic Information Services with the abiding interest and rights as follows:

- This Project/Thesis is the sole legal property of Universiti Malaysia Sarawak (UNIMAS).
- The Centre for Academic Information Services has the lawful right to make copies for the purpose of academic and research only and not for other purpose.
- The Centre for Academic Information Services has the lawful right to digitalise the content for the Local Content Database.
- The Centre for Academic Information Services has the lawful right to make copies of the Project/Thesis for academic exchange between Higher Learning Institute.
- No dispute or any claim shall arise from the student itself neither third party on this Project/Thesis once it becomes the sole property of UNIMAS.
- This Project/Thesis or any material, data and information related to it shall not be distributed, published or disclosed to any party by the student except with UNIMAS permission.

Student signature _____ (Date) 25/6/2015

Supervisor signature: _____ (Date) 25/6/2015

Current Address:
No 226 Jalan Seragam, United Garden, Jalan Kelang Lama, ~~58000~~ 58200 Kuala Lumpur.

Notes: * If the Project/Thesis is **CONFIDENTIAL** or **RESTRICTED**, please attach together as annexure a letter from the organisation with the period and reasons of confidentiality and restriction.

[The instrument is duly prepared by The Centre for Academic Information Services]

SYSTEMATICS OF *HIPPOSIDEROS GALERITUS* USING CYTOCHROME C OXIDASE I GENE, MORPHOLOGY AND ECHOLOCATION CALL

TAN JO ANN

A thesis submitted in fulfilment of the requirement for the degree of
Bachelor of Science with Honours
(Animal Resource Science and Management)

Faculty of Resource Science and Technology
Department of Zoology
UNIVERSITI MALAYSIA SARAWAK
2015

Declaration

I hereby declare that no portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification to this or any other university or institute of higher learning.

TAN JO ANN

Animal Resource Science and Management

Department of Zoology

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

Acknowledgment

I would like to express my greatest gratitude to my supervisor Dr. Faisal Ali Anwarali Khan and co - supervisor Ms. Roberta Chaya Tawie anak Tingga for all the valuable knowledge, experiences, encouragement and kind guidance throughout my journey in completing my thesis. My gratitude also goes to Mr. Chuah Kee Man, Mr. Isham Azhar and my mentor Mr. Charlie Justin Mergie Laman in providing valuable advices along the way which had motivated me to work beyond my limits. I am also grateful for the assistances provided from postgraduate students; Ms. Sultana Parvin, Ms. Nurul Farah Diyana and the rest members of the mammal's research team in providing a conducive work environment, team spirit and guidance throughout in field and in the laboratory. My appreciation is also extended to the members of Zoology Department; Mr. Mohd Ridwan Abd Rahman and Mr. Huzal Irwan Husin for their expertise and generous support that greatly assisted the research. I would also like to thank Sarawak Forestry Department and Sarawak Forestry Corporation to grant my research permit with the number, NCCD.907.4.4(JLD.11)-11 so that I could carry out this research in Protected Areas. I am indeed grateful and would also like to acknowledge the following research grants; Research Acculturation Grants Scheme: RAGS/STWN109(2)/1043/2013(10) from Ministry of Higher Education (MoHE) and Niche Research Grant Scheme Subproject 2: NRGS/1088/2013 (02) from the Ministry of Education, both had greatly supported my research at UNIMAS. Last but not least to my parents whom had supported me financially and was the backbone of my support.

Table of Contents

List of Abbreviations

Defined below are abbreviations that appear in the text.

BNP	Bako National Park
ddH_2O	Double distilled water
dNTP	Deoxyribonucleotide triphosphate
$MgCl_2$	Magnesium chloride
MNP	Mulu National Park
NNP	Niah National Park
SEA	Southeast Asia
SFR	Sabal Forest Reserve
TDNP	Tanjung Datu National Park

List of Tables

List of Figures

List of Appendices

Systematics of *Hipposideros galeritus* using Cytochrome C Oxidase I Gene, Morphology and Echolocation call

Tan Jo Ann

Animal Resource Science and Management
Department of Zoology
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak

Abstract

The taxonomy of *Hipposideros galeritus* remain unresolved as it does not clearly define evolutionary relationships boundaries between species of the same genus. Previous molecular phylogenetic studies revealed the instances of paraphyly and deep genetic divergence within currently recognised *H. galeritus* lineage indicating diversity within this species is underrepresented with current nomenclature. Hence, this study aims to resolve the taxonomy of *H. galeritus* complex using cytochrome c oxidase I gene (COI), morphometric and echolocation call data. The significant genetic variation (2.9 - 18.4 %), indicates possible different subspecies and distinct genetic species. Sabah and Northern Sarawak clade suggest to be *H. labuanensis,* Malay Peninsula as *H. galeritus galeritus* while high genetic distance and distinct monophyletic clades; Indochina and Southern Sarawak with Niah highly suggest new species nomenclature which is currently unrecognized. Potential subspecies is also seen to occur within same biogeographic region supported with notable genetic distances. Morphometric data analysed as DFA in SPSS shows DBC as the best resolving character. Echolocation calls were analysed with Kaleidoscope and DFA. The variables of FMaxE and FMEAN had significant difference between Sarawak populations but not able to classify populations. There is no relationship between forearm length and peak frequency in this study. More representative samples should be included from biogeographic regions (apart from Sarawak) to have a full understanding of *H. galeritus* taxonomy. A more vigorous documentation of echolocation and morphometric data will be helpful to have a complete description of *H. galeritus* taxonomy.

Keywords: Cytochrome c oxidase I, echolocation, genetic divergence, *Hipposideros galeritus*, systematics.

Abstrak

Taksonomi Hipposideros galeritus tidak dapat diselesaikan dengan jelas bagi menentukan sempadan evolusi antara spesis di dalam genus yang sama. Sebelumnya kajian molekul filogenetik mendedahkan kewujudan 'paraphyly' dan perbezaan genetik yang besar dalam salasilah H. galeritus. Ini menunjukkan kepelbagaian H. galeritus kurang diwakili dengan tatanama semasa. Oleh itu, kajian ini bertujuan untuk menyelesaikan taksonomi H. galeritus kompleks dengan menggunakan gen sitokrom c oxidase (COI), morfometrik dan panggilan gema. Variasi genetik yang besar (2.9 % - 18.4 %), menunjukkan kemungkinan subspesies dan spesies genetik yang berbeza. Klad Sabah dan Utara Sarawak dicadang mewakili H. labuanensis, Semenanjung Tanah Melayu sebagai H. galeritus galeritus manakala jarak genetik yang tinggi dan klad monophyletik berbeza; Indochina dan Selatan Sarawak dengan Niah mencadangkan penamaan spesies baru yang kini tidak diiktiraf. Subspesies juga berpotensi berlaku di kawasan biogeografi yang sama disokong oleh jarak genetik yang ketara. Data morfometrik telah dianalisis dalam DFA menggunakan SPSS. Hasil kajian menunjukkan DBC sebagai pembolehubah terbaik dalam penyelasian morfometrik. Panggilan ekolokasi dianalisis dengan Kaleidoscope dan DFA. Pemboleh ubah FMaxE dan FMEAN mempunyai perbezaan signifikasi antara populasi Sarawak tetapi tidak berjaya mengelaskan populasi. Panjan lengan H. galeritus dan frekuensi puncak tidak mempunyai hubungan. Lebih banyak sampel perlu dikaji dari kawasan biogeografi (selain daripada Sarawak) untuk mempunyai pemahamam penuh taksonomi H. galeritus. Dokumentasi terperinci panggilan gema dan morfometrik akan membantu dalam deskripsi taksonomi H. galeritus.

Kata Kunci: Sitokrom c oxidase I, panggilan gema, perbezaan genetik, Hipposideros galeritus, systematik.

1.0 Introduction

Bats from the order Chiroptera form a monophyletic group within the class Mammalia (Baker *et al.*, 1991). Members of this order have been previously proposed to have independently evolved into two separate lineages, recognised as the Suborder Megachiroptera (non-echolocating bats except genus *Rousettus*) and Microchiroptera (echolocating bats) (Baker *et al.*, 1991). However, recent molecular studies have proposed two new Suborders namely Yinpterochiroptera that includes both echolocating and non echolocating bats (comprising of Pteropodidae, Rhinolophidae, Hipposideridae, Megadermatidae, Rhinopomatidae and Craseonycteridae) and Yangochiroptera (remaining echolocating bat families) (Jones & Teeling, 2006). This finding highlights that echolocation probably have evolved independently within currently recognised bat families.

Of these families, member from the family Hipposideridae are the most widespread and ecologically diverse group (Murray *et al.*, 2012). Hipposideridae consists of two genus in Borneo namely *Hipposideros* and *Coelops* (Payne *et al.*, 2007). Recent records account for a total of 73 species from the genus *Hipposideros* that are distributed in the old world tropics (Simmons, 2005). Of this, a total of 11 species are found in Borneo (Payne *et al.*, 2007).

Hipposideros galeritus or commonly known as Cantor's roundleaf bat is an example of a bat species from the genus *Hipposideros*. This species is placed as a subgroup under the *bicolor* species group (Hill, 1963). This species is widespread from Sri Lanka, India, Borneo, Java, Thailand and Peninsular Malaysia (Payne *et al.*, 2007) with the type locality in Penang island (Hill, 1963).

This species is selected for the proposed study, as recent documentation reveals deep genetic divergence within *H. galeritus* clade which holds the potential to harbour biologically distinct species (Francis *et al.*, 2010). In addition, the presence of cryptic species in this family is common and biodiversity of *H. galeritus* could be underestimated (Murray *et al.*, 2012).

Cryptic species living in sympatry will experience microhabitat variation. Hence, detail study of cryptic species will certainly show difference in their measurements of the cranial, external morphology and echolocation call frequency. Adjustment towards these measurements and call frequency is needed in order to adapt with various ecological attributes and as a force of natural selection (Sazali & Aziz, 2012). Morphology adjustment either internal or external is logical to affect echolocation call frequency since it affects capture success on specific insects as food source. Thus, documentation and comparison of morphometrics and acoustic divergence could serve as an additional useful tool in identifying cryptic species. Recent study only documented morphometrics of *H. galeritus* in selected localities in Sarawak but no comparisons was made between individuals from different geographic distributions nor validated by molecular work (Sazali & Aziz, 2012).

Cytochrome c oxidase I (COI) mitochondrial gene (mtDNA) was used in this research to resolve the taxonomy complex of *H. galeritus*. COI is also known as a DNA barcoding tool and has a slow rate of change in their gene sequences which is suitable to be used for species level identification (Hebert *et al.*, 2003). However the rate of substitution is also fast enough to act as a suitable gene candidate to differentiate individuals (Hebert *et al.*, 2003). This gene has been demonstrated to be effective for species identification and individual differentiation

of several bats species in Southeast Asia (SEA) (Francis *et al.*, 2010). Thus, the use of multifaceted approach will be certainly important to accurately access species diversity in SEA.

1.1 Problem Statement

The taxonomy of *H. galeritus* is unstable as it had undergone changes from being a member of separate group in Tate (1941) classification while considered as a subgroup in more recent literatures (Hill, 1963; Simmons, 2005). These classifications of groups are based on morphology. It is not known if these morphological similarities reflect evolutionary relationship within the genus *Hipposideros* as great similarities in morphological terms make classification difficult (Hill, 1963). Having said that, three primary divisions of *Hipposideros* are proposed which allocate three general morphology criteria but all three groups showed independent evolutionary trends (Hill, 1963). Although these primary divisions of *Hipposideros* had been recovered lately, their relationship remains equivocal (Murray *et al.*, 2012). Hence, in the proposed research, the classification of *H. galeritus* along with its intraspecific and interspecific relationships in the phylogenetic tree will be described based on *H. galeritus* genetic divergence.

Molecular genetics studies reveal instances of paraphyly of *H. galeritus* from Sulawesi indicating that the diversity of this genus is underrepresented with current nomenclature and needs to be validated by echolocation call and extensive sampling (Murray *et al.*, 2012). Furthermore, the recent study by Francis *et al.* (2010) using COI and Murray *et al.* (2012) using ND2 gene, both had shown deep genetic divergence within *H. galeritus* of selected localities but did not further discuss its taxonomic implications. High genetic divergence of a bat species signifies potential different biological species (Baker and Bradley, 2006) and could be identified by acoustic divergence (Kingston *et al.*, 2001). The number of samples used in previous study is not sufficient to give a strong validation of *H. galeritus* divergence

and phylogenetic relationships according to geographic locations. Thus, this study was carried out to overcome the problem of cryptic species by documenting morphology, echolocation and genetics based on biogeographic regions using all accessible data. This study hope to strengthen the taxonomic position of this species.

1.2 Objective

This study aims to;

1. identify all the available *H. galeritus* genetic lineages through COI mtDNA marker gene.
2. determine the echolocation call variation among all the available *H. galeritus* genetic lineages.
3. determine the morphological variation among all the available *H. galeritus* genetic lineages.

1.3 Hypothesis

Genetic divergence

H_0: There is no significance difference in genetic divergence of *H. galeritus* populations.

H_1: There is significance difference in genetic divergence of *H. galeritus* populations.

Echolocation call

H_0: There is no significance difference between echolocation call frequencies from selected localities.

H_1: There is significance difference between echolocation call frequencies from selected localities.

Morphology

H_0: There is no significance difference in morphology from selected localities.

H_1: There is significance difference in morphology from selected localities.

1.4 Significance of Study

Study on the systematics of *H. galeritus* can give an insight towards bats' evolution, acoustic diversity, species assemblages and taxonomy. It is important to carry out this study as bats' diversity is the second largest in the mammalian order (Neuweiler, 1990). Evidence of cryptic diversity calls for the need to extend species identification through multiple methods such as skull morphology, external morphology, echolocation call frequency and genetics sequencing studies (Neuweiler, 1990). At present, several bats species from genus *Hipposideros* are identified as new species among the named population with some similar works done as described by Thabah *et al.* (2006).

The proposed study would serve as a reference point to make appropriate management decisions for effective conservation planning in the future. In addition, results from this study is a source of valuable data to the tree of life and as a reference for systematicians, taxonomists and subsequent researchers toward a more detailed description of *H. galeritus* species.

2.0 Literature review

2.1 Biogeography of Southeast Asia (SEA)

Sunda Shelf is a shallow continent that was joined as Borneo, Sumatra and Java and surrounded by lesser islands (Heaney, 1986). The Sunda Shelf was flooded during interglacial period (rise of sea level) and was exposed occasionally as dry land during glacial period of Pleistocene (Heaney, 1986). In the past, more than 90 % of the time, Sundaland was 1.5 – 2.0 times larger than it is now due to mean sea level being 62 m lower than those today (Woodruff, 2010). Thus, almost a two-fold increase or decrease in land area in respective to sea level changes (Woodruff, 2010). Climate was cooler and large forests area and savannah covered the expose lands (Woodruff, 2010). Thus, forest expansion and contraction occur in accordance with oscillations in land area makes up the biogeographic regions (Woodruff, 2010).

Thailand is located as a boundary between Indochinese and Sundaland provinces. These provinces contained distinct climate, flora and fauna composition due to the migratory route at Kra Isthmus, Thailand which is favoured only at low sea levels (Tougard, 2001). When sea levels are low, faunal exchanges within Sundaland itself occur as land bridges emerge acting as migratory route for animals and Thailand is part of the continental migratory route (Tougard, 2001). Despite this faunal exchange, at the Late Middle Pleistocene period, mammal faunas from mainland displayed modern character whereas fauna composition at Java contained endemic forms (Tougard, 2001). In addition to that, climatic cooling occurred at the northern hemisphere during Pleistocene period had caused the movement of northern

faunas to the south (Tougard, 2001). Thus, this indicates the initial separation or isolation of species that could not cope with climatic cooling, had migrated south while those which can adapt to the environment did not migrate. Thus, it is deduce that over the years, genetic divergence would have occurred and it is reasonable to check between the Northern and Southern regions of Kra Isthmus, as it is hypothesised to have high genetic divergence between these two areas. In addition, since Thailand is part of the continental migratory route, it is hypothesised that Thailand will contain similar genetic divergence to the northern part of Peninsular Malaysia. Sunda Shelf includes Java, Sri Lanka and India where *H. galeritus* can be found today and at present illustrated as discontinues distribution. Thus it is likely that genetic divergence of *H. galeritus* is also prominent when compared between these areas.

2.2 Taxonomy and Morphological Variation Within *H. galeritus* Complex

H. galeritus has broad, rounded base and triangular pointed ears (Hill, 1963; Payne *et al.*, 2007). Typically, *H. galeritus* have fur colour of dark grey-brown and pinkish grey noseleaf colouration (Payne *et al.*, 2007). Male *H. galeritus* had facial glands located behind the posterior noseleaf (Khan *et al.*, 2008) while in female there is a sunken region with collection of hair at the base (Hill, 1963). *Hipposideros galeritus* has two well-developed lateral supplementary leaflets (Payne *et al.*, 2007).

In comparison of external morphology, *H. cervinus* and *H. coxi* are very similar to *H. galeritus* as there are presences of two lateral leaflets (Payne *et al.*, 2007). However, *H. cervinus* could be distinguished by a narrower median noseleaf as compared to the posterior

noseleaf whereas *H. coxi* has a larger noseleaf which completely covers the muzzle. Tail length is another character that distinguishes *H. galeritus* (30 - 43 mm) from *H. cervinus* (21 - 28 mm) (Payne *et al.*, 2007). However tail length difference is very small and at times could have overlapping measurements due to variation. Forearm of *H. galeritus* recorded at a range of 47 - 51 mm with weight 6.3 - 8.5 g in Borneo (Payne *et al.*, 2007).

The skull morphology of *H. galeritus* is small, elongated with low sagittal crest (Hill, 1963). The bridge between maxillae and premaxillae is V-shaped while the palatation is U-shaped. There is no supraorbital ridge. The zygomata is slender with width less than the mastoid width. The sphenoidal bridge is medium sized, not to cover or hide the lateral apertures (Hill, 1963). This skull description however resembled a lot of *H. ater*. Thus, it is suggested that the *H. galeritus* skull may had been mismatched with the skin specimen (Tate, 1941). However, recent examination of *H. galeritus* skull from Peninsular Malaysia and Thailand had shown clear cranial differences as compared to *H. ater* skull (Jenkins & Hill, 1981). Hence, the suggestion of mismatched skull and skinned specimen is incorrect (Jenkins & Hill, 1981).

For dental morphology, the upper incisors have two lobes which are weakly shaped while upper canines are thin with low cusps facing outwards (Hill, 1963). The front upper premolar is very discrete and seen to be extruded from the toothrow (Hill, 1963). The anterior lower premolar measures one third to one half of length and height of second lower premolar (Hill, 1963).